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Review article

Immunity in the female sheep reproductive tract

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Abstract – Immune surveillance in the female reproductive tract is dependent on the interplay of many factors that include the expression of pattern recognition receptors on epithelial cells, resident leukocyte populations and hormones, none of which are uniform. The lower reproductive tract must accommodate the presence of commensal organisms whereas the upper reproductive tract is sterile. However, the upper female reproductive tract has its own immunological challenge in that it must tolerate the presence of a semi-allogeneic fetus if pregnancy is to succeed. So, immune activation and effector mechanisms to control pathogens may be qualitatively and quantitatively different along the reproductive tract. Our knowledge of innate and adaptive immunity in the sheep is less comprehensive than that of human or mouse. Nevertheless, comparative studies suggest that there are likely to be conserved innate immune sensory mechanisms (e.g. Toll-like receptors) and defence mechanisms (anti-proteases, defensins) that combine to limit infection in its early stages while shaping the adaptive response that leads to immunological memory and long-term protection. There are many pathogens that target the reproductive tract, and in particular the placenta, where specialised immunoregulatory mechanisms are operational. Among such pathogens are bacteria belonging to the genera *Chlamydia*/*Chlamydophila* that chronically infect the reproductive tracts of sheep and humans and ultimately cause disease through inflammation and tissue damage. An understanding of the immunological microenvironment of the reproductive tract is important for the design of novel control strategies to control chlamydial disease.

reproduction / sheep / *Chlamydia* / abortion / pregnancy

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1. INTRODUCTION

Mucosal sites inhabited by commensal flora need to be capable of discriminating between commensals and the pathogenic organisms they encounter in order to generate protective immune responses. The lower female reproductive tract is one such site, whereas the upper reproductive tract is normally sterile [66]. These differences in microbial exposure along the reproductive tract suggest that the innate defence mechanisms required to sense and respond to micro-organisms are unlikely to be uniform from vagina to uterus [79]. This is exemplified by the fact that organisms that are harmless (or even beneficial) to the host at one anatomical site can cause disease when introduced to another.

The innate immune system performs three important functions: firstly, it prevents infection through physical means; secondly, should infection occur, it produces anti-microbial compounds designed to limit pathogen multiplication until the adaptive response has time to develop; and thirdly, it produces immunomodulatory molecules that drive the phenotype of the adaptive immune response. This combination of defence mechanisms is necessary for the host to protect itself from a wide range of infectious agents.

The most notable infectious diseases of the reproductive tract of sheep are those that infect the placenta and cause abortion. These can be of viral (Border disease virus), bacterial (*Chlamydophila abortus*, *Salmonella* spp., *Campylobacter* spp., *Listeria* spp., *Brucella* spp.), rickettsial (*Coxiella burnetii*) or protozoal (*Toxoplasma gondii*) aetiology [52]. Some of these abortifacient agents are zoonotic and therefore pose a risk to human health and are of comparative medical inter-

est. In many cases they cause abortion when the ewe is infected for the first time during pregnancy, and although not causing severe clinical disease in the ewes, are fatal for the fetus. The stage of gestation at which exposure occurs often affects the outcome of infection. However, in certain cases, notably *C. abortus*, infection can be established prior to pregnancy and clinically manifest itself only when ewes abort. There are two important points to establish at the outset. The first is that although these pathogens cause disease in the reproductive tract, their route of transmission is most commonly oro-nasal rather than venereal [52]. This indicates that the extra-uterine immune response following primary infection is not sufficient to prevent systemic dissemination to the placenta. The second is that disease manifests itself in the placenta and not at other anatomical sites. This is likely to be reflective of the specialised immune environment of the pregnant uterus necessary to accommodate the semi-allogeneic fetus which may not be compatible with the type of immune response necessary for pathogen control.

This review will draw on our current knowledge of the physiology and immunology of the female reproductive tract of sheep, integrate this with comparative information from other species and discuss how the various factors contribute to disease pathogenesis and reproductive failure.

2. REPRODUCTION IN FEMALE SHEEP

2.1. Anatomy of the reproductive tract

The reproductive tract of the ewe consists of a vestibule leading to the vagina,

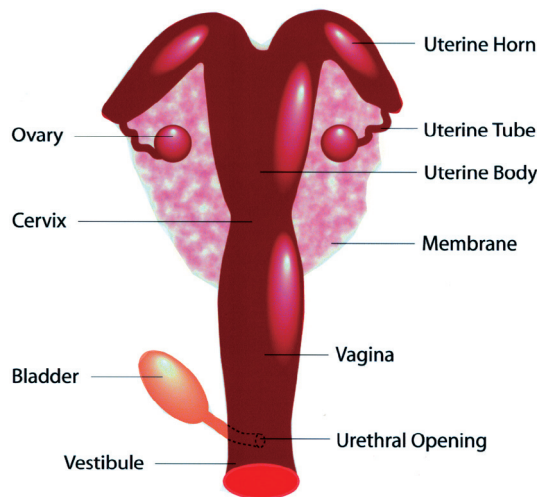


Figure 1. Schematic view of the female sheep reproductive system.

separated from the bipartite uterus by the cervix, with the uterus linked to the ovaries by the uterine tubes (Fig. 1). The urethra joins the reproductive tract at the base of the vagina and marks the boundary with the vestibule, making the vestibule common to both the reproductive and urinary tracts. The gross anatomical differences between the lower and upper reproductive tracts are reflected in the differences in the mucous membranes that line them. The vagina has a stratified squamous epithelium whereas the glandular mucosa that lines the uterus (the endometrium) has a stratified columnar epithelium. The endometrium of the sheep has approximately 90 caruncles. Caruncles are protruding cup-like structures that act as sites of attachment for the fetal cotyledons of the placenta during pregnancy. The uterus is glandular, with uterine glands distributed around the endometrium, except in the caruncles.

2.2. The ovine placenta

The ovine placenta is synepitheliochorial, meaning that the fetal chorion makes direct contact with the uterine epithelium [81]. There is less invasion of the maternal tissue by fetal cells in this type of placenta than in the hemochorial placenta of humans and some rodent species. However, some

ovine fetal trophoblast cells from the cotyledonary villi in the placentome do fuse with maternal cells in the endometrium to form characteristic binucleate cells. Exchange of nutrients and waste products between the dam and fetus occurs in the placentomes, structures comprised of a cotyledon and a caruncle. The placentomes contain interdigitating villi to increase the surface area between fetus and ewe (Fig. 2).

2.3. Reproductive hormones

Reproduction is an energy-demanding process, particularly for females. It is therefore advantageous for female mammals to conceive at times that result in their young being born with the best chance of survival. The reproductive pattern is dictated by hormones, and the period when females are sexually receptive is known as oestrus. Sheep are polyoestrus, which means that they can have successive oestrus cycles during their breeding season. The number of cycles varies between breeds, ranging from 1–20, and appears to be linked to the climate to which the breed is adapted, ensuring lambs are born in the most favourable conditions. The average oestrus cycle in ewes is around 17 days, with oestrus itself (ovulation) lasting 24–48 h. The levels of various hormones vary in the plasma during

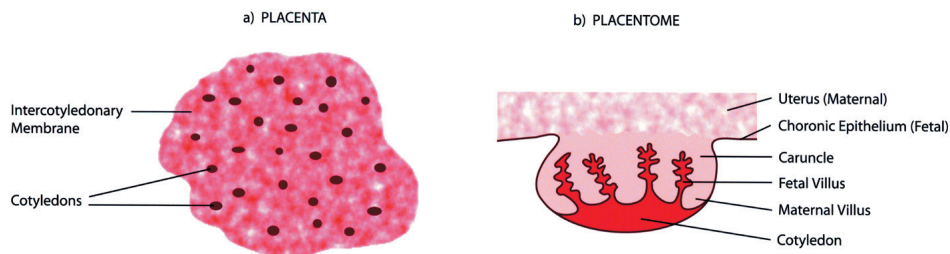


Figure 2. (a) The cotyledonary placenta of sheep at parturition. (b) The placentome structure at it exists in utero. Fetal cotyledons attach to caruncles on the maternal uterine epithelium to form the placentomes. The villi interdigitate to facilitate exchange of nutrients and waste materials.

the cycle. Oestrus is preceded by an increase in follicle-stimulating hormone, which decreases as oestrogen levels rise, promoting a surge of luteinising hormone which then leads to ovulation. Progesterone levels are at their lowest around ovulation. If the ovum that is released is fertilised, an embryo will begin to develop in the uterus. At this stage it is important for the fertilised ovum to develop, and also for the ewe to stop producing further ova. To this end, the embryo secretes products that inhibit uterine secretion of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). $PGF_{2\alpha}$ is a product of the non-pregnant uterus that causes leutolysis and regression of the corpus luteum, a structure in the ovary that produces high levels of progesterone. Progesterone inhibits further oestrus cycles and promotes attachment and development of the embryo, thereby allowing pregnancy to progress. This process is known as maternal recognition of pregnancy. In some species the corpus luteum is the primary source of progesterone throughout pregnancy. However, in the ewe after day 50 of gestation the placenta itself secretes sufficient progesterone to maintain pregnancy (the gestation period of sheep is around 147 days). Plasma levels of progesterone increase markedly in ewes between days 90 and 125 gestation, then drop sharply before parturition. Levels of oestrogen increase from around day 120 and show a sharp rise 24 h prior to parturition. Levels of prostaglandin E_2 (PGE_2) are also greatly elevated in the latter stages of pregnancy and around parturition [28, 81].

Some of these hormones are known to directly influence immune function, notably oestrogen, progesterone and PGE_2 . Fluctuations in hormone levels during the reproductive cycle and pregnancy are therefore of considerable interest when considering host immune control of infectious disease in the reproductive tract [4]. The effects of hormones on immune function will be discussed in detail in Section 4.

3. INNATE IMMUNITY IN THE REPRODUCTIVE TRACT

3.1. Physical barriers to infection

Physical barriers form the first line of host defence at mucosal surfaces. For example, tight epithelial junctions and mucosal secretions are important factors in preventing microbial access to tissues. Vaginal commensals such as *Lactobacillus* spp. produce lactic acid and hydrogen peroxide to create a low (< 5) pH environment. This helps to protect the lower reproductive tract from pathogenic micro-organisms and thereby reduces the possibility of infection reaching the upper reproductive tract [66]. Nevertheless, most pathogens that enter the body do so via mucosal surfaces, so the host needs to be able to identify and respond rapidly and appropriately to limit the spread of infection.

3.2. Pathogen recognition

It had long been considered that the innate response was non-specific whereas specificity for host immunity was conferred by the adaptive response, with lymphocytes, and in particular CD4⁺ T cells, dictating how the host responded. This paradigm has shifted somewhat in recent years with the discovery of pattern recognition receptors (PRR). PRR (e.g. the Toll-like receptors; TLR) respond to generic pathogen-associated molecular patterns (PAMP) such as lipopolysaccharide (LPS), flagellin, unmethylated oligodeoxynucleotides (ODN), double-stranded RNA and heat shock proteins (hsp). Ligation of PRR by PAMP elicits distinct cytokine profiles by responding cells which confers a previously unrecognised level of specificity to the innate response [37, 48]. These recognition pathways are prophylactically important since they can potentially be exploited by carefully-designed delivery strategies to drive the adaptive response to candidate vaccine antigens in an appropriate direction.

TLR are transmembrane proteins that exhibit specificity for distinct PAMP. The intracellular signalling domain of TLR resembles that of the interleukin-1 receptor. Binding of the specific PAMP to the TLR initiates a cascade which leads to activation and translocation of nuclear transcription factors that result in distinct patterns of expression of immune-related genes [75].

Eleven TLR are expressed in humans, between them detecting a wide range of products from bacteria, viruses and protozoa [37, 48]. TLR have been cloned in a wide variety of experimental and domestic species, including mice, chickens, pigs, dogs and cats. To date no TLR homologues have been cloned in the sheep, but TLR have been cloned in cattle [78]. Immunohistochemical analyses have revealed the expression of TLR 1, 2, 3, 5 and 6 throughout the human reproductive tract, whilst TLR 4 is only found in the upper tract [24]. This supports a previous report of decreased

expression of mRNA encoding TLR4, but not TLR2, in the lower reproductive tract compared to the upper tract [64]. TLR4, in conjunction with CD14 and MD-2, is thought to be the principal pathway through which cells recognise bacterial LPS [58]. This has implications for mucosal sites that harbour commensal Gram-negative microorganisms. Tolerance of commensal microflora by the gut mucosal epithelium can be achieved by a down-regulation of TLR4 and MD-2 expression [2]. It has also been proposed that the failure of epithelial cells to express CD14 is to prevent the host mounting unnecessary responses to commensal Gram-negative bacteria [22]. Interestingly, human uterine epithelial cells express the mRNA encoding TLR 1–9 and respond to TLR agonists (with the exception of LPS) by releasing pro-inflammatory cytokines [68, 69]. Human uterine epithelial cells also require soluble CD14 to respond effectively to LPS, even though the uterus is normally sterile [34].

Choriocarcinoma cell lines derived from human placenta express TLR 1–10 [47]. TLR2 and TLR4 protein expression in human placenta is strongest on the trophoblasts that cover the peripheral chorionic villi and which constitute the immediate barrier between mother and fetus [35]. Trophoblasts can produce cytokines following TLR4 ligation, but undergo apoptosis following TLR2 ligation [1].

Collectively, these studies indicate that there is the potential for microbial detection in both the lower and upper reproductive tracts, but with some qualitative differences. This suggests that micro-organisms can elicit different responses along the reproductive tract and may even influence fetal survival during gestation.

3.3. Defensins

Epithelial cells of the female reproductive tract not only have the potential to recognise pathogens, but they are also capable of responding to infection by producing

antimicrobial compounds, chemokines and cytokines. Among the antimicrobial compounds are the defensins, a highly-conserved group of cationic peptides that exhibit a broad spectrum of activity against bacteria, fungi and viruses [29]. The two main defensin sub-families are the α - and β -defensins. The α -defensins are produced mainly by neutrophils and Paneth cells whilst the β -defensins are produced by epithelial cells and keratinocytes [29, 33]. Six human β -defensins have so far been identified. Of these, human β -defensins (HBD)-1-4 are expressed in the endometrium, although their expression can be regulated by different stimuli [44]. For example, expression of HBD-2 and HBD-3 can be up-regulated in endometrial cells by pro-inflammatory cytokines such as IL-1 β plus TNF- α or by IFN- γ [42, 45]. HBD-1 is constitutively expressed by epithelial cells at several mucosal sites whereas HBD-2 is inducible by LPS [5, 77].

In addition to their antimicrobial roles, β -defensins also have immunomodulatory properties. HBD-2 binds the chemokine receptor CCR6, thereby acting as a chemoattractant for immature dendritic cells and memory T cells [82], whereas murine β -defensin-2 binds TLR-4 on dendritic cells and can initiate inflammatory host immune responses [7].

Two β -defensins have been identified in the ovine gastrointestinal tract and these have been termed SBD-1 and -2 [38]. There are no reports of α -defensins in sheep. Similar to the expression pattern of HBD-1, SBD-1 is constitutively expressed in epithelial cells throughout the gastrointestinal tract. SBD-2 is expressed in the gut, lung and uterus [3, 56]. It is not yet possible to say if and how SBD-1 or -2 contribute to innate immune defence in the reproductive tract of sheep or how their expression is regulated. However, it is known that SBD expression in ovine lung is modulated by pathogens. *Mannheimia haemolytica* down-regulates SBD-1 and -2 expression [3], whereas parainfluenza virus up-regulates

SBD-1 expression [30]. This indicates that reproductive pathogens may have a similar modulatory effect to promote their survival.

3.4. Anti-proteases

Cells within human female reproductive tract produce the anti-protease molecules elafin and secretory leukocyte protease inhibitor (SLPI). Both elafin and SLPI neutralise the elastases that are produced by infiltrating neutrophils during infection and are thought to be important in regulating tissue damage as a result of the inflammatory process. In addition, both molecules exhibit antimicrobial properties and therefore contribute to innate host defence. Ovine orthologues of SLPI and elafin have been recently cloned. Both molecules are induced by LPS challenge in the ovine lung and possess anti-elastase properties [8, 9]. The direct anti-microbial properties of ovine SLPI and elafin remain to be established and expression of ovine SLPI and elafin within the ovine female reproductive tract not been determined. However given the similarity in the expression patterns at other mucosal sites compared with other species it would be highly surprising if SLPI or elafin were not components of the innate immune strategy of the ovine female reproductive tract.

SLPI is expressed by cell lines derived from various sections of the human female reproductive tract, including the vagina, cervix, endometrium and decidua, as well as trophoblasts in the placenta [25, 41]. Elafin is expressed by epithelial cells in the human vagina and endometrium [43, 63]. Like defensins, expression of mRNA encoding for elafin in primary human endometrial cells is up-regulated by IL-1 β plus TNF- α [43]. However, the same cytokine stimulus does not increase mRNA encoding SLPI in primary human endometrial cells, but does in endometrial cell lines, highlighting the importance of careful data interpretation from in vitro observations [42].

3.5. Other innate antimicrobial defence mechanisms

There are other molecules expressed in the human female reproductive tract that have antimicrobial activity and also chemotactic activity. Such molecules are likely to be important bridges between innate and adaptive immune responses and will be of interest to define in sheep. For example, the stratified squamous epithelium of the human vagina produces surfactant protein A (SP-A), a collagenous lectin formally thought to be restricted to lung [79]. SP-A is antimicrobial, influences cytokine production and is involved in chemotaxis. CCL20/macrophage inflammatory protein (MIP) 3 α is a chemokine produced by human uterine epithelial cells. It is a ligand of CCR6, the receptor that also binds human β -defensins, suggesting homology between CCL20 and the human β -defensins. Consistent with this, CCL20 exhibits anti-microbial activity [36].

4. FEMALE REPRODUCTIVE HORMONES AND INNATE IMMUNITY

Hormones play a very important role in regulating host immunity in the genital tract, distinguishing this from other mucosal sites [39]. There is a vast body of literature on the influence of female hormones on cell populations, antigen presentation and cell function in the reproductive tracts of humans and mice (see [80] for review), but not in sheep. Hormone levels fluctuate during the reproductive cycle, influencing immune surveillance and disease susceptibility. For example, HBD expression in the endometrium is differentially regulated by the menstrual cycle and by the oral contraceptive pill [26, 44]. Expression of SLPI also fluctuates during the menstrual cycle, increasing in human epithelial cells in response to progesterone or in rat uterus in response to oestrogen [14, 46, 79]. Although hormones dominate the reproductive tract environment, not all products of epithelial cells are modulated by hormones, so there is a selective effect. For example, unlike many other

products of epithelial cells of the female reproductive tract, expression of SP-A is not influenced by hormones and remains constitutive [51]. Studies on hormonal influences in the reproductive tract in sheep have focussed primarily on pregnancy. This will be discussed in Section 6.

5. ADAPTIVE IMMUNITY IN THE REPRODUCTIVE TRACT

Humoral and cellular adaptive immune effector mechanisms operate in the reproductive tract. Immunoglobulin A (IgA) and IgG are found in uterine and vaginal secretions [80]. The normal healthy female genital tract harbours few T cells, but infection with *Chlamydia trachomatis* results in recruitment of CD4+ve and CD8+ve T cells [39]. The same study also found a unique population of T cells in the mouse uterus that appear to perform a regulatory role rather than a protective role. These cells are TCR $\alpha\beta$ +ve, CD3+ve, CD4-ve, CD8-ve, inhibit proliferation of splenic T cells and are extrathymically-derived, since they are found in nude mice. It is thought that they represent a highly specialised population of T cells with a particular function in the female genital tract, but this remains to be fully defined [39]. They are not the same as conventional CD4+veCD25+ve regulatory T (Treg) cells, key control elements of the adaptive immune response that suppress auto-reactive T cells and prevent inflammation-mediated tissue damage [71]. Some broad "signature" characteristics of Treg cells are expression of the transcription factor FoxP3 and the production of IL-10 and TGF- β , two cytokines that exert immunosuppressive effects [74].

6. PREGNANCY AND HOST IMMUNITY

6.1. Accommodation of the semi-allogeneic fetus

It was recognised by Peter Medawar over fifty years ago that mammalian pregnancy

Table I. Specialised immunological features at the materno-fetal interface that promote successful pregnancy. These observations are drawn from several species and it a matter of speculation if they are operational in sheep.

Immune modulator	Effect
Fetal/trophoblast	
Lack of expression of classical MHC class I or MHC class II molecules	Evade recognition by maternal $\alpha\beta$ T cells
Expression of non-classical MHC class I molecules	Evade lysis by maternal NK cells
Expression of Fas ligand	Induce apoptosis of Fas-bearing maternal T cells and neutrophils
Expression of IDO	Tolerise maternal T cells
Production of IL-10 and TGF- β	Down-regulate maternal immune reactivity
Production of hormones	Favour maternal Th2-type immunity
No 'Danger' signal	No activation of maternal immunity
Maternal	
Uterine NK cells/ $\gamma\delta$ T cells	Control trophoblast invasion
Absent/low expression of IL-2, TNF- α and IFN- γ	Minimise inflammatory cell activation, promotion of trophoblast survival
Hormone production	Bias maternal immunity away from an inflammatory phenotype
Production of GM-CSF, CSF-1	Promote placental development

was not compatible with the self-nonsel model of immune activation, and that adaptations of maternal immunity and/or specialised immunological characteristics of the placenta allow survival of the semi-allogeneic fetus [53]. This paradox has still not been fully resolved, but we do know that the specialised immunological and physiological interactions at the materno-fetal interface are complex. The structure of the placenta is central to successful pregnancy, yet it is not uniform across eutherian mammals, reflective of differences in gestation periods, litter sizes and number of mates (genetic diversity) between species [6]. Some key immunological features of mammalian pregnancy are listed in Table I. Note that these have been derived principally from studies in humans and mice. Their existence in sheep and relative contribution to the success of ovine pregnancy remains largely unknown, and inter-species comparisons must be drawn with care [6, 18].

6.2. Uterine lymphocyte populations

The pregnant sheep uterus has an increased number of intraepithelial CD8+ve/ $\gamma\delta$ T cell receptor (TCR) +ve large granular leukocytes (LGL) compared to the non-pregnant uterus [54]. These cells are more granular in the pregnant uterus, suggesting a state of activation that is reminiscent of the granular CD56^{bright} NK cells that have been postulated to perform an important role in limiting trophoblast invasion in the human hemochorial placenta [57]. Supportive of this hypothesis, ovine uterine CD8+ve/ $\gamma\delta$ TCR+ve LGL express perforin, suggesting a cytolytic function [27]. Both $\gamma\delta$ TCR+ve LGL and NK cells are found in human decidua [17, 55]. Fluctuations in the number of uterine LGL may be due, at least in part, to hormone production. Progesterone and oestradiol induce a pattern of chemokine expression in human endometrium that is consistent with the fluctuating numbers of

uterine NK cells during the menstrual cycle [70]. The number of $\gamma\delta$ TCR+ve intraepithelial LGL in the ovine uterus drops dramatically within days of parturition. This decrease is due to a combination of cell migration, apoptosis and degranulation, suggesting that they have completed their function in the inter-placentomal areas of the uterine epithelium during pregnancy [59]. Both progesterone and a uterine serine proteinase inhibitor have been postulated to have immunoregulatory roles in the pregnant ovine uterus [32, 76]. Inhibition of the cytolytic activity of ovine endometrial cells by the uterine serine proteinase inhibitor may be necessary to protect the mother from the potentially invasive fetus while allowing sufficient contact for nutrient and waste exchange [76].

An unequivocal description of NK cells in sheep has remained elusive, so it has been impossible to ascribe a definitive function during ovine pregnancy. A monoclonal antibody (Mab) produced against a molecule expressed on cytotoxic cells of fish (NK5C6, anti-Function Associated Molecule; anti-FAM) but which reacts with rodent and human NK cells, has been found to inhibit the activity of cytotoxic cells derived from ovine endometrium [76]. However, NK5C6 does not react with ovine peripheral blood leukocytes (PBL) in flow cytometry or detect leukocytes in ovine endometrium by immunocytochemistry. These conflicting data may be in part due to the isotype of NK5C6. It is an IgM Mab, an isotype that is technically more difficult to work with than IgG Mab. Reagents and probes that identify NK cell receptors in cattle have recently been developed which may be applicable in sheep. Cattle have genes that encode killer cell immunoglobulin like receptors (KIR), CD94 (a killer cell lectin-like receptor) and NKp46 (an activating receptor expressed exclusively on NK cells in the human) [72]. A Mab produced against recombinant bovine NKp46 reacts with cytotoxic bovine PBL [73]. However, it is not yet known if this Mab detects uterine

NK cells in cattle, or if it cross-reacts with ovine PBL.

6.3. Regulatory T cells

Treg cells can regulate allo-responses and are of great interest as therapeutic targets in transplantation medicine. It is therefore not surprising that Treg cells have now become a focus of attention in reproductive immunology and tolerance of the semi-allogeneic fetus. Expansion of the Treg population has been found in both the uterus and the maternal periphery during pregnancy. Mouse models indicate a role for Treg in preventing maternal rejection of semi-allogeneic, but not syngeneic, fetuses [67, 83]. Oestrogen increases both the number of splenic CD25+ve T cells and FoxP3 expression within those cells in mice, indicating that Treg cells are affected by the hormonal environment [65]. Treg have not yet been defined in sheep.

7. CHLAMYDIAL INFECTION IN THE REPRODUCTIVE TRACT

Chlamydia/Chlamydophila are obligate intracellular Gram-negative bacteria that cause a variety of diseases in many hosts [50]. With regard to infection of the reproductive tract, two of these organisms stand out. *Chlamydia trachomatis* is the most common diagnosed sexually-transmitted infectious agent in humans in the UK¹ while *Chlamydophila abortus* is by far the single most common cause of infectious ovine abortion reported annually in the UK². Both organisms can cause inapparent primary infections that then result in reproductive failure as they persist. Chronic *C. trachomatis* infection prevents fertilization or implantation as a result of inflammation and

¹ Health Protection Agency, Chlamydia (*Chlamydia trachomatis*) [on line] (2005) http://www.hpa.org.uk/infections/topics_az/hiv_and_sti/sti-chlamydia/chlamydia.htm.

² Veterinary Laboratories Agency, [on line] (2005) <http://www.defra.gov.uk/corporate/vla/science/science-vida03b.htm>.

scarring of the upper reproductive tract whereas *C. abortus* causes abortion associated with inflammation and tissue destruction of the placenta. *C. abortus* persists in the non-pregnant ewe (at a site as yet unidentified), but then can be found in the placenta from around day 90 gestation of the pregnancy subsequent to infection. Abortion occurs around day 125–135 gestation, which is typically 2–3 weeks before the end of the normal gestation period of 147 days [12].

The susceptibility of epithelial cells in the female reproductive tract to *C. trachomatis* is not uniform during the reproductive cycle. Pig cervical epithelial cells isolated in the early (oestrogen-dominant) phase of the cycle are around ten times more susceptible than cells isolated later in the cycle (progesterone-dominant) [31]. It has been proposed that *C. trachomatis* may exploit the oestrogen receptor to facilitate entry into host cells [16]. It is not known if *C. abortus* utilizes this mechanism in sheep, but if so, it would help to explain the observations of increased shedding of *C. abortus* during estrus in infected ewes [62].

7.1. Host immunity

For both *C. abortus* and *C. trachomatis*, protective host immunity is dependent on a Th1-type response dominated by IFN- γ production. IFN- γ induces intracellular expression of indoleamine 2,3-dioxygenase (IDO), a tryptophan-degrading enzyme. Since *C. abortus* and *C. trachomatis* are auxotrophic for tryptophan, they cannot multiply if IDO is activated [10, 21]. It is worth noting that the hormonal changes associated with pregnancy and oestrus do not favour the production of IFN- γ . This may allow a persistent or latent extra-uterine chlamydial infection to recrudescence and invade the placenta [18].

Sheep develop clinically protective immunity to *C. abortus* after abortion, such that repeat abortions are rare [20]. This does not necessarily reflect induction of sterile immunity in the ewe, since infectious organisms can be recovered from the vagina during estrus of sheep that have previously aborted [62].

7.2. Route of infection

As previously mentioned, although *C. abortus* causes reproductive disease, the common route of transmission to susceptible sheep is oro-nasal, the most likely source of infection being contaminated placentas of aborting ewes. Nevertheless, sexual transmission cannot be ruled out. Experimental vaginal inoculation of ewes prior to breeding can cause abortion whereas subcutaneous inoculation does not [61]. However, infection by either the vaginal or subcutaneous route in ewes that are pregnant results in abortion. Moreover, infection by the subcutaneous route prior to breeding actually protects against abortion in sheep re-challenged during pregnancy. These observations indicate that *C. abortus* infection of non-pregnant sheep by a mucosal route induces a qualitatively different response to that elicited by subcutaneous infection, the former allowing the establishment of a persistent infection that manifests itself by causing abortion in a subsequent pregnancy.

Chlamydiae can activate early innate immune responses via PAMP ligation of PRR to influence disease progression. Oviduct pathology is significantly reduced in TLR2 knockout (but not TLR4 knockout) mice infected with *C. trachomatis*. Moreover, ligation of TLR4 by whole organisms appears to have an anti-inflammatory effect [15]. Chlamydial hsp60 can activate cells through ligation of TLR4 [11], whereas *C. trachomatis* LPS is recognised by TLR2 [23]. Activation via TLR2 but not TLR4 in human trophoblasts can lead to apoptosis in vitro [1]. If this occurs during placental infection with *C. abortus*, the resulting damage to the placenta could compromise the pregnancy. PRR expression at the site of primary infection can therefore influence how the host responds, which may be the case in sheep infected by *C. abortus* by different routes. This question can be addressed when reagents become available to investigate PRR expression in the reproductive tract of sheep.

7.3. Mechanism of abortion

The exact cause of abortion as a result of *C. abortus* infection is not known. Our current knowledge points to a combination of factors that include damage to the placentomes, destruction of the chorionic epithelium, hormone imbalance, maternal leukocyte infiltration of the uterus, fetal mononuclear cell placental infiltration, and placental thrombosis as a consequence of placental expression of inflammatory mediators such as TNF- α that are incompatible with successful pregnancy [13, 18, 60].

There is a notable paradox regarding chlamydial abortion. IDO is constitutively expressed in both human and mouse trophoblast (see Tab. I), despite both species being susceptible to abortion as a result of natural (human) or experimental (mouse) placental infection with *C. abortus* [22, 40]. Since the function of IDO in the placenta is to degrade tryptophan and tolerise maternal T cells to paternal allo-antigens, one would expect this to be an inhospitable site for *C. abortus*, but it clearly is not. However, IDO expression in trophoblast varies dependent on the stage of pregnancy [19]. This could explain why *C. abortus* can target the placenta at particular stages of pregnancy and raises important questions about immune regulation at the materno-fetal interface in sheep. As yet, ovine IDO has not been cloned so it is not known what role, if any, it plays in the syneptheliochorial placenta of sheep or in regulation of T cell immunity in general in ruminants. Such information will be very valuable in building a more complete picture of the pathogenesis of ovine chlamydial abortion and inform on the design of new control strategies.

8. CONCLUSIONS AND FUTURE PROSPECTS

Infectious diseases of the reproductive tract in sheep, particularly those that cause abortion, impact on animal health, welfare, production and the economy worldwide.

However, the fact that many of these abortifacient agents reach the uterus and placenta systemically following primary infection at a remote mucosal site rather than by venereal transmission suggests that control by non-mucosal vaccine delivery should be a viable prophylactic option. This is indeed the case. To take *C. abortus* as an example, parenteral administration of live-attenuated or whole-killed organisms protect sheep against abortion [49]. Nevertheless, ovine chlamydial abortion remains a problem. The reasons for this are complex, and are not just related to immune responses, but involve facets of animal management systems and diagnostic surveillance that have resulted in poor uptake of the available vaccines. Sub-unit vaccines and alternative delivery systems could circumvent these problems.

One would predict that a combination of mucosal and systemic immunity would provide the best protection against *C. abortus* infection in sheep. This would ideally involve antigen delivery via a mucosal surface such as the reproductive tract. By selectively targeting components of the innate immune system, the induction of an appropriate adaptive memory response could be achieved. A greater understanding of the innate and adaptive elements operational in the female reproductive tract in sheep is required for such a rational approach to vaccine design. We can draw on existing comparative knowledge of human and mouse reproductive tract immunology to prioritise research into those molecules that are likely to play a role. Conversely, evaluation of chlamydial vaccines in sheep will inform on suitable strategies for human chlamydial vaccines, of which there are currently none.

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REFERENCES

- [1] Abrahams V.M., Bole-Aldo P., Kim Y.M., Straszewski-Chavez S.L., Chaiworapongsa T., Romero R., Mor G., Divergent trophoblast responses to bacterial products mediated by TLRs, *J. Immunol.* 173 (2004) 4286–4296.
- [2] Abreu M.T., Vora P., Faure E., Thomas L.S., Arnold E.T., Arditu M., Decreased expression of Toll-like receptor-4 and MD-2 correlates with intestinal epithelial cell protection against dysregulated proinflammatory gene expression in response to bacterial lipopolysaccharide, *J. Immunol.* 167 (2001) 1609–1616.
- [3] Ackermann M.R., Gallup J.M., Zabner J., Evans R.B., Brockus C.W., Meyerholz D.K., Grubor B., Brogden K.A., Differential expression of sheep beta-defensin-1 and -2 and interleukin 8 during acute *Mannheimia haemolytica* pneumonia, *Microb. Pathog.* 37 (2004) 21–27.
- [4] Beagley K.W., Gockel C.M., Regulation of innate and adaptive immunity by the female sex hormones oestradiol and progesterone, *FEMS Immunol. Med. Microbiol.* 38 (2003) 13–22.
- [5] Becker M.N., Diamond G., Verghese M.W., Randell S.H., CD14-dependent lipopolysaccharide-induced beta-defensin-2 expression in human tracheobronchial epithelium, *J. Biol. Chem.* 275 (2000) 29731–29736.
- [6] Billington W.D., The immunological problem of pregnancy: 50 years with the hope of progress. A tribute to Peter Medawar, *J. Reprod. Immunol.* 60 (2003) 1–11.
- [7] Biragyn A., Ruffini P.A., Leifer C.A., Klyushnenkova E., Shakhov A., Chertov O., Shirakawa A.K., Farber J.M., Segal D.M., Oppenheim J.J., Kwak L.W., Toll-like receptor 4-dependent activation of dendritic cells by beta-defensin 2, *Science* 298 (2002) 1025–1029.
- [8] Brown T.I., Mistry R., Collie D.D., Tate S., Sallenave J.M., Trappin ovine molecule (TOM), the ovine ortholog of elafin, is an acute phase reactant in the lung, *Physiol. Genomics* 19 (2004) 11–21.
- [9] Brown T.I., Mistry R., Gray R., Imrie M., Collie D.D., Sallenave J.M., Characterization of the ovine ortholog of secretory leuko-protease inhibitor, *Mamm. Genome* 16 (2005) 621–630.
- [10] Brunham R.C., Rey-Ladino J., Immunology of *Chlamydia* infection: implications for a *Chlamydia trachomatis* vaccine, *Nat. Rev. Immunol.* 5 (2005) 149–161.
- [11] Bulut Y., Faure E., Thomas L., Karahashi H., Michelsen K.S., Equils O., Morrison S.G., Morrison R.P., Arditu M., Chlamydial heat shock protein 60 activates macrophages and endothelial cells through Toll-like receptor 4 and MD2 in a MyD88-dependent pathway, *J. Immunol.* 168 (2002) 1435–1440.
- [12] Buxton D., Barlow R.M., Finlayson J., Anderson I.E., Mackellar A., Observations on the pathogenesis of *Chlamydia psittaci* infection of pregnant sheep, *J. Comp. Pathol.* 102 (1990) 221–237.
- [13] Buxton D., Anderson I.E., Longbottom D., Livingstone M., Wattegedera S., Entrican G., Ovine chlamydial abortion: characterization of the inflammatory immune response in placental tissues, *J. Comp. Pathol.* 127 (2002) 133–141.
- [14] Chen D., Xu X., Cheon Y.P., Bagchi M.K., Bagchi I.C., Estrogen induces expression of secretory leukocyte protease inhibitor in rat uterus, *Biol. Reprod.* 71 (2004) 508–514.
- [15] Darville T., O'Neill J.M., Andrews C.W. Jr., Nagarajan U.M., Stahl L., Ojcius D.M., Toll-like receptor-2, but not Toll-like receptor-4, is essential for development of oviduct pathology in chlamydial genital tract infection, *J. Immunol.* 171 (2003) 6187–6197.
- [16] Davis C.H., Raulston J.E., Wyrick P.B., Protein disulfide isomerase, a component of the estrogen receptor complex, is associated with *Chlamydia trachomatis* serovar E attached to human endometrial epithelial cells, *Infect. Immun.* 70 (2002) 3413–3418.
- [17] Engelhardt H., King G.J., Uterine natural killer cells in species with epitheliochorial placentation, *Nat. Immun.* 15 (1996) 53–69.
- [18] Entrican G., Immune regulation during pregnancy and host-pathogen interactions in infectious abortion, *J. Comp. Pathol.* 126 (2002) 79–94.
- [19] Entrican G., IDO: a crossroads of immunology and physiology? *J. Reprod. Immunol.* 61 (2004) 63–65.
- [20] Entrican G., Buxton D., Longbottom D., Chlamydial infection in sheep: immune control versus fetal pathology, *J. R. Soc. Med.* 94 (2001) 273–277.
- [21] Entrican G., Wattegedera S., Chui M., Oemar L., Rocchi M., McInnes C., Gamma interferon fails to induce expression of indoleamine 2,3-dioxygenase and does not control the growth of *Chlamydia abortus* in BeWo

- trophoblast cells, *Infect. Immun.* 70 (2002) 2690–2693.
- [22] Entrican G., Wattegedera S., Rocchi M., Fleming D.C., Kelly R.W., Wathne G., Magdalenic V., Howie S.E., Induction of inflammatory host immune responses by organisms belonging to the genera *Chlamydia*/*Chlamydophila*, *Vet. Immunol. Immunopathol.* 100 (2004) 179–186.
- [23] Erridge C., Pridmore A., Eley A., Stewart J., Poxton I.R., Lipopolysaccharides of *Bacteroides fragilis*, *Chlamydia trachomatis* and *Pseudomonas aeruginosa* signal via Toll-like receptor 2, *J. Med. Microbiol.* 53 (2004) 735–740.
- [24] Fazeli A., Bruce C., Anumba D.O., Characterization of Toll-like receptors in the female reproductive tract in humans, *Hum. Reprod.* 20 (2005) 1372–1378.
- [25] Fichorova R.N., Anderson D.J., Differential expression of immunobiological mediators by immortalized human cervical and vaginal epithelial cells, *Biol. Reprod.* 60 (1999) 508–514.
- [26] Fleming D.C., King A.E., Williams A.R., Critchley H.O., Kelly R.W., Hormonal contraception can suppress natural antimicrobial gene transcription in human endometrium, *Fertil. Steril.* 79 (2003) 856–863.
- [27] Fox A., Meeusen E., Sheep perforin: identification and expression by gamma delta T cells from pregnant sheep uterine epithelium, *Vet. Immunol. Immunopathol.* 68 (1999) 293–296.
- [28] Frandson R.D., Wilke W.L., Fails A.D., *Anatomy and physiology of farm animals*, 6th ed., Lippincott Williams and Wilkins, 2003.
- [29] Ganz T., Defensins: antimicrobial peptides of innate immunity, *Nat. Rev. Immunol.* 3 (2003) 710–720.
- [30] Grubor B., Gallup J.M., Meyerholz D.K., Crouch E.C., Evans R.B., Brogden K.A., Lehmkuhl H.D., Ackermann M.R., Enhanced surfactant protein and defensin mRNA levels and reduced viral replication during parainfluenza virus type 3 pneumonia in neonatal lambs, *Clin. Diagn. Lab. Immunol.* 11 (2004) 599–607.
- [31] Guseva N.V., Knight S.T., Whittimore J.D., Wyrick P.B., Primary cultures of female swine genital epithelial cells in vitro: a new approach for the study of hormonal modulation of *Chlamydia* infection, *Infect. Immun.* 71 (2003) 4700–4710.
- [32] Hansen P.J., Regulation of uterine immune function by progesterone – lessons from the sheep, *J. Reprod. Immunol.* 40 (1998) 63–79.
- [33] Harder J., Meyer-Hoffert U., Wehkamp K., Schwichtenberg L., Schroder J.M., Differential gene induction of human beta-defensins (hBD-1, -2, -3, and -4) in keratinocytes is inhibited by retinoic acid, *J. Invest. Dermatol.* 123 (2004) 522–529.
- [34] Hirata T., Osuga Y., Hirota Y., Koga K., Yoshino O., Harada M., Morimoto C., Yano T., Nishii O., Tsutsumi O., Taketani Y., Evidence for the presence of Toll-like receptor-4 system in the human endometrium, *J. Clin. Endocrinol. Metab.* 90 (2005) 548–556.
- [35] Holmlund U., Cebers G., Dahlfors A.R., Sandstedt B., Bremme K., Ekstrom E.S., Scheynius A., Expression and regulation of the pattern recognition receptors Toll-like receptor-2 and Toll-like receptor-4 in the human placenta, *Immunology* 107 (2002) 145–151.
- [36] Hoover D.M., Boulegue C., Yang D., Oppenheim J.J., Tucker K., Lu W., Lubkowski J., The structure of human macrophage inflammatory protein-3alpha/CCL20. Linking antimicrobial and CC chemokine receptor-6-binding activities with human beta-defensins, *J. Biol. Chem.* 277 (2002) 37647–37654.
- [37] Hopkins P.A., Sriskandan S., Mammalian Toll-like receptors: to immunity and beyond, *Clin. Exp. Immunol.* 140 (2005) 395–407.
- [38] Huttner K.M., Brezinski-Caliguri D.J., Mahoney M.M., Diamond G., Antimicrobial peptide expression is developmentally regulated in the ovine gastrointestinal tract, *J. Nutr.* 128 (1998) 297S–299S.
- [39] Johansson M., Lycke N., A unique population of extrathymically derived alpha beta TCR+CD4-CD8- T cells with regulatory functions dominates the mouse female genital tract, *J. Immunol.* 170 (2003) 1659–1666.
- [40] Kerr K., Entrican G., McKeever D., Longbottom D., Immunopathology of *Chlamydophila abortus* infection in sheep and mice, *Res. Vet. Sci.* 78 (2005) 1–7.
- [41] King A.E., Critchley H.O., Kelly R.W., Presence of secretory leukocyte protease inhibitor in human endometrium and first trimester decidua suggests an antibacterial protective role, *Mol. Hum. Reprod.* 6 (2000) 191–196.
- [42] King A.E., Fleming D.C., Critchley H.O., Kelly R.W., Regulation of natural antibiotic expression by inflammatory mediators and mimics of infection in human endometrial

- epithelial cells, *Mol. Hum. Reprod.* 8 (2002) 341–349.
- [43] King A.E., Critchley H.O., Sallenave J.M., Kelly R.W., Elafin in human endometrium: an antiprotease and antimicrobial molecule expressed during menstruation, *J. Clin. Endocrinol. Metab.* 88 (2003) 4426–4431.
- [44] King A.E., Critchley H.O., Kelly R.W., Innate immune defences in the human endometrium, *Reprod. Biol. Endocrinol.* 1 (2003) 116.
- [45] King A.E., Fleming D.C., Critchley H.O., Kelly R.W., Differential expression of the natural antimicrobials, beta-defensins 3 and 4, in human endometrium, *J. Reprod. Immunol.* 59 (2003) 1–16.
- [46] King A.E., Morgan K., Sallenave J.M., Kelly R.W., Differential regulation of secretory leukocyte protease inhibitor and elafin by progesterone, *Biochem. Biophys. Res. Commun.* 310 (2003) 594–599.
- [47] Klaffenbach D., Rascher W., Rollinghoff M., Dotsch J., Meissner U., Schnare M., Regulation and signal transduction of Toll-like receptors in human chorioncarcinoma cell lines, *Am. J. Reprod. Immunol.* 53 (2005) 77–84.
- [48] Liew F.Y., Xu D., Brint E.K., O'Neill L.A., Negative regulation of Toll-like receptor-mediated immune responses, *Nat. Rev. Immunol.* 5 (2005) 446–458.
- [49] Longbottom D., Chlamydial vaccine development, *J. Med. Microbiol.* 52 (2003) 537–540.
- [50] Longbottom D., Coulter L.J., Animal chlamydioses and zoonotic implications, *J. Comp. Pathol.* 128 (2003) 217–244.
- [51] MacNeill C., Umstead T.M., Phelps D.S., Lin Z., Floros J., Shearer D.A., Weisz J., Surfactant protein A, an innate immune factor, is expressed in the vaginal mucosa and is present in vaginal lavage fluid, *Immunology* 111 (2004) 91–99.
- [52] Martin W.B., Aitken I.D., *Diseases of sheep*, 3rd ed., 2000, Blackwell Science, Oxford, 512 p.
- [53] Medawar P.B., Some immunological and endocrinological problems raised by the evolution of viviparity in vertebrates, *Symp. Soc. Exp. Biol. Med.* 7 (1953) 320–338.
- [54] Meeusen E., Fox A., Brandon M., Lee C.S., Activation of uterine intraepithelial gamma delta T cell receptor-positive lymphocytes during pregnancy, *Eur. J. Immunol.* 23 (1993) 1112–1117.
- [55] Meeusen E.N., Bischof R.J., Lee C.S., Comparative T-cell responses during pregnancy in large animals and humans, *Am. J. Reprod. Immunol.* 46 (2001) 169–179.
- [56] Meyerholz D.K., Gallup J.M., Grubor B.M., Evans R.B., Tack B.F., McCray P.B. Jr., Ackermann M.R., Developmental expression and distribution of sheep beta-defensin-2, *Dev. Comp. Immunol.* 28 (2004) 521–530.
- [57] Moffett-King A., Natural killer cells and pregnancy, *Nat. Rev. Immunol.* 2 (2002) 656–663.
- [58] Mukhopadhyay S., Herre J., Brown G.D., Gordon S., The potential for Toll-like receptors to collaborate with other innate immune receptors, *Immunology* 112 (2004) 521–530.
- [59] Nasar A., Rahman A., Meeusen E.N., Lee C.S., Peri-partum changes in the intraepithelial lymphocyte population of sheep interplacental endometrium, *Am. J. Reprod. Immunol.* 47 (2002) 132–141.
- [60] Navarro J.A., Garcia de la Fuente J.N., Sanchez J., Martinez C.M., Buendia A.J., Gutierrez-Martin C.B., Rodriguez-Ferri E.F., Ortega N., Salinas J., Kinetics of infection and effects on the placenta of *Chlamydomphila abortus* in experimentally infected pregnant ewes, *Vet. Pathol.* 41 (2004) 498–505.
- [61] Papp J.R., Shewen P.E., Pregnancy failure following vaginal infection of sheep with *Chlamydia psittaci* prior to breeding, *Infect. Immun.* 64 (1996) 1116–1125.
- [62] Papp J.R., Shewen P.E., Gartley C.J., Abortion and subsequent excretion of chlamydiae from the reproductive tract of sheep during estrus, *Infect. Immun.* 62 (1994) 3786–3792.
- [63] Pfundt R., van Ruissen F., Vlijmen-Willems I.M., Alkemade H.A., Zeeuwen P.L., Jap P.H., Dijkman H., Fransen J., Croes H., van Erp P.E., Schalkwijk J., Constitutive and inducible expression of SKALP/elafin provides anti-elastase defense in human epithelia, *J. Clin. Invest.* 98 (1996) 1389–1399.
- [64] Pioli P.A., Amiel E., Schaefer T.M., Connolly J.E., Wira C.R., Guyre P.M., Differential expression of Toll-like receptors 2 and 4 in tissues of the human female reproductive tract, *Infect. Immun.* 72 (2004) 5799–5806.
- [65] Polanczyk M.J., Carson B.D., Subramanian S., Afentoulis M., Vandenbark A.A., Ziegler S.F., Offner H., Cutting edge: estrogen drives expansion of the CD4+CD25+ regulatory T cell compartment, *J. Immunol.* 173 (2004) 2227–2230.

- [66] Quayle A.J., The innate and early immune response to pathogen challenge in the female genital tract and the pivotal role of epithelial cells, *J. Reprod. Immunol.* 57 (2002) 61–79.
- [67] Saito S., Sasaki Y., Sakai M., CD4(+)CD25^{high} regulatory T cells in human pregnancy, *J. Reprod. Immunol.* 65 (2005) 111–120.
- [68] Schaefer T.M., Desouza K., Fahey J.V., Beagley K.W., Wira C.R., Toll-like receptor (TLR) expression and TLR-mediated cytokine/chemokine production by human uterine epithelial cells, *Immunology* 112 (2004) 428–436.
- [69] Schaefer T.M., Fahey J.V., Wright J.A., Wira C.R., Innate immunity in the human female reproductive tract: antiviral response of uterine epithelial cells to the TLR3 agonist poly(I:C), *J. Immunol.* 174 (2005) 992–1002.
- [70] Sentman C.L., Meadows S.K., Wira C.R., Eriksson M., Recruitment of uterine NK cells: induction of CXC chemokine ligands 10 and 11 in human endometrium by estradiol and progesterone, *J. Immunol.* 173 (2004) 6760–6766.
- [71] Shevach E.M., CD4⁺ CD25⁺ suppressor T cells: more questions than answers, *Nat. Rev. Immunol.* 2 (2002) 389–400.
- [72] Storset A.K., Slettedal I.O., Williams J.L., Law A., Dissen E., Natural killer cell receptors in cattle: a bovine killer cell immunoglobulin-like receptor multigene family contains members with divergent signaling motifs, *Eur. J. Immunol.* 33 (2003) 980–990.
- [73] Storset A.K., Kulberg S., Berg I., Boysen P., Hope J.C., Dissen E., NKp46 defines a subset of bovine leukocytes with natural killer cell characteristics, *Eur. J. Immunol.* 34 (2004) 669–676.
- [74] Taams L.S., Akbar A.N., Peripheral generation and function of CD4⁺CD25⁺ regulatory T cells, *Curr. Top. Microbiol. Immunol.* 293 (2005) 115–131.
- [75] Takeda K., Akira S., Toll-like receptors in innate immunity, *Int. Immunol.* 17 (2005) 1–14.
- [76] Tekin S., Hansen P.J., Natural killer-like cells in the sheep: functional characterization and regulation by pregnancy-associated proteins, *Exp. Biol. Med.* (Maywood.) 227 (2002) 803–811.
- [77] Vora P., Youdim A., Thomas L.S., Fukata M., Tesfay S.Y., Lukasek K., Michelsen K.S., Wada A., Hirayama T., Arditì M., Abreu M.T., Beta-defensin-2 expression is regulated by TLR signaling in intestinal epithelial cells, *J. Immunol.* 173 (2004) 5398–5405.
- [78] Werling D., Jungi T.W., Toll-like receptors linking innate and adaptive immune response, *Vet. Immunol. Immunopathol.* 91 (2003) 1–12.
- [79] Wira C.R., Fahey J.V., The innate immune system: gatekeeper to the female reproductive tract, *Immunology* 111 (2004) 13–15.
- [80] Wira C.R., Grant-Tschudy K.S., Crane-Godreau M.A., Epithelial cells in the female reproductive tract: a central role as sentinels of immune protection, *Am. J. Reprod. Immunol.* 53 (2005) 65–76.
- [81] Wooding F.B.P., Flint A.P.F., Placentation, in: *Marshall's Physiology of Reproduction, Pregnancy and Lactation*, 4th ed., Vol. 3, 1999, pp. 235–429.
- [82] Yang D., Chertov O., Bykovskaia S.N., Chen Q., Buffo M.J., Shogan J., Anderson M., Schroder J.M., Wang J.M., Howard O.M., Oppenheim J.J., Beta-defensins: linking innate and adaptive immunity through dendritic and T cell CCR6, *Science* 286 (1999) 525–528.
- [83] Zenclussen A.C., CD4(+)CD25⁺ T regulatory cells in murine pregnancy, *J. Reprod. Immunol.* 65 (2005) 101–110.